CSIRO PUBLISHING

Reproduction, Fertility and Development, 2021, **33**, 381–391
https://doi.org/10.1071/RD20274

Review

Physiological implications of COVID-19 in reproduction: angiotensin-converting enzyme 2 a key player

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Abstract. The COVID-19 outbreak, caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), was first identified in China, and it has quickly become a global threat to public health due to its rapid rate of transmission and fatalities. Angiotensin-converting enzyme 2 (ACE2) has been identified as a receptor that mediates the entry of SARS-CoV-2 into human cells, as in the case of severe acute respiratory syndrome coronavirus (SARS-CoV). Several studies have reported that ACE2 expression is higher in Leydig, Sertoli and seminiferous ductal cells of males, as well as in ovarian follicle cells of females, suggesting possible potential pathogenicity of the coronavirus in the reproductive system. Higher ACE2 expression in the human placenta and reports of vertical transmission of SARS-CoV-2 among clinical cases have increased the relevance of further studies in this area. This review focuses on the interaction between SARS-CoV-2 and the ACE2 receptor and speculates on the mechanistic interplay in association with male and female reproductive physiology. In addition, based on the available literature, we discuss the alleged sex differences in terms of the infectivity of SARS-CoV-2, which is claimed greater among males, and further explore the physiological role of ACE2 and 17β-oestradiol for the same.

Keywords: COVID-19, SARS-CoV-2, ACE2 receptors, reproductive physiology, gender bias.

Received 16 October 2020, accepted 19 January 2021, published online 18 March 2021

Introduction

The ongoing global COVID-19 pandemic first emerged in Wuhan, China, in December 2019. COVID-19 is caused by a novel strain of coronavirus, namely severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which belongs to Family Coronaviridae. Coronaviruses are known to cause illness in both animals and humans. From the physiological point of view, the study of SARS-CoV-2 infection is interesting because the virus uses angiotensin converting enzyme (ACE) 2 receptors to enter host cells after priming of the spike protein of SARS-CoV-2 by transmembrane protease serine-2 (TMPRSS2; Hoffmann *et al.* 2020). The versatile nature of ACE2 receptors and their widespread distribution in the host body have perplexed the research community while addressing the pathogenesis of SARS-CoV-2 inside the body. However, the extensive distribution of ACE2 in the testes, ovary, placenta, and associated cells has drawn the attention of many researchers in recent times to gain an understanding of their role in changes in a host's fertilising competence during infection with SARS-CoV-2.

Epidemiological data pertaining to infections with severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) indicate that the frequency of cases and deaths are higher among men than women (Karlberg *et al.* 2004; Channappanavar *et al.* 2017). The reason for male vulnerability to coronavirus may be explained by a clear sex prejudice (as detailed in the 'Sex differences in COVID-19 infections' section below) connected to scientific, social, and cultural roots, lifestyle differences and reproductive hormones. COVID-19 impact-related sex-disaggregated data is important for reducing health inequities

in reproductive medicine. Although the global data show sex differences in COVID-19 susceptibility and mortality, systematic studies are lacking. Thus, this review discusses the physiological and biochemical effects of COVID-19 on reproductive physiology and explores the possible role of ACE2 receptors and reproductive hormones in the alleged sex differences in infection. An understanding of these issues may provide novel insights into the treatment of COVID-19 patients, with a special emphasis on reproductive health issues.

Possible effects of COVID-19 on the male reproductive system

The importance of studying the effects of COVID-19 on male reproduction arises from the fact that ACE2 is highly expressed in the testis, in both germ and somatic cells (Shen *et al.* 2020; Wang and Xu 2020). The expression of furin domains in the epididymis of men supports the risk of localisation of SARS-CoV-2 in the male reproductive tract (Thimon *et al.* 2006). Moreover, the transmembrane protein TMPRSS2, used for S-protein priming by the virus, is highly expressed in the epithelial cells of the prostate lumen (Chen *et al.* 2010) and orchitis was reported as a postinfection complication of the previous SARS-CoV outbreak in China in 2002 (Xu *et al.* 2006). Although feline coronavirus was reported to cause orchitis in cats (Sigurðardóttir *et al.* 2001), there are no reports regarding the association between orchitis and SARS-CoV-2.

Paoli *et al.* (2020) reported the absence of viral RNA in the seminal fluid of a man with a history of COVID-19 infection. However, the latest research letter from Li *et al.* (2020*a*) drew attention to the reporting of positive SARS-CoV-2 semen samples in severe COVID-19 cases, which signifies the possible infection of the testes and other reproductive structures by COVID-19. A viral breach of the immune-privileged environment of the testis may cause inflammation and an autoimmune attack of sperm cells, leading to infertility. A recent study reported a significant increase in serum LH concentrations and a marked decrease in the serum testosterone to LH ratio, thus resulting in subclinical or compensated hypogonadism in male COVID-19 patients (Pozzilli and Lenzi 2020). It is also possible that although a COVID-19 infection may no longer appear active, the virus may continue its replication silently, finally breaking through the blood-testes barrier, leading to the establishment of pathogenesis in the otherwise secure and controlled microenvironment of the testes.

Testicular ACE2

ACE2 is a versatile enzyme that is mostly associated with cardiovascular and renal functions; however, the distribution of ACE2 in the testes and reproductive cells indicates its substantial involvement in the regulation of the fertilising competence of spermatozoa. Gianzo *et al.* (2018) demonstrated the presence of ACE2 receptors located on the human sperm equatorial segment using immunofluorescence. ACE2 activity was found to be 13-fold higher in the sperm plasma membrane than in seminal plasma (Shibahara *et al.* 2001); in addition, ACE2 activity is higher in post- than prepubertal testes (Douglas *et al.* 2004). The wider distribution of the ACE2 receptors in testicular tissues may be used by SARS-CoV-2, and may result in serious outcomes in terms of the regulation of sperm fertilising competence. Furthermore, the involvement of the local renin–angiotensin system (RAS) in male reproduction was identified by the presence of several RAS components (i.e. prorenin, renin, angiotensinogen, angiotensin (Ang) I, AngII, ACE and ACE2) in the testes and epididymides of humans and other mammals (Langford *et al.* 1993; Fuchs *et al.* 2005; Deguchi *et al.* 2007).

There are two ACE isoenzymes in the testis, namely the somatic (sACE) and testicular (tACE) isoforms, whereas the testicular and somatic isoforms of ACE2 are identical. Among all the tissues in the body, the testes have nearly the highest levels of ACE2 mRNA and protein expression (Fan *et al.* 2021). Interestingly, the testicular expression of ACE2 is age related and is highest in men in their 30s and lowest in men in their 60s. These findings suggest a higher susceptibility of younger men to COVID-19, probably due to higher ACE2 expression.

Proposed mechanistic pathways of SARS-COV-2 and testicular disruption of spermatogenesis

Even though no studies have been reported regarding the impairment of spermatogenesis during COVID-19 infection, an interplay of different cascades that may be involved in disrupting the spermatogenic cycle inside the testes during COVID-19 infection can be proposed.

The most likely mechanism is disruption of the normal spermatogenesis pathway by SARS-COV-2, facilitated by ACE2 receptors, by targeting the RAS and its associated pathways (Fig. 1). ACE2 is the main Ang-(1–7)-forming enzyme, either through the hydrolysis of the potent vasoconstrictor AngII (Vickers *et al.* 2002) or the hydrolysis of AngI to inactive Ang-(1–9), which is subsequently converted to Ang-(1–7) by ACE (Donoghue *et al.* 2000; Burrell *et al.* 2004; Rice *et al.* 2004). Ang-(1–7) is reported to act on the Mas-related G-protein-coupled receptors (MRGPRS), thereby antagonising the biological effects of AngII. Bradykinin (BK), one of the most important peptides regulating water and ion balance in the body, is a part of the kinin–kallikrein system. In the testes, BK has been reported to have roles in prespermatogonial cell proliferation *in vitro* (Atanassova *et al.* 1998), anion secretion in the epididymis and vas deferens (Cheuk *et al.* 2002; Pierucci-Alves and Schultz 2008), smooth muscle contraction and prostaglandin production in the vas deferens (Peredo and Celuch 2001), as well as to affect the motility and vitality of ejaculated spermatozoa (Somlev and Subev 1998). The effects of ACE2 in the male reproductive system of humans have been demonstrated by the severe impairment of spermatogenesis in infertile subjects who have lower levels of ACE2, Ang-(1–7) and MRGPRS compared with fertile subjects (Reis *et al.* 2010). Further, the localisation of ACE2 in Leydig cells and a marked reduction in testes weight in Mas-deficient mice (Leal *et al.* 2009) provide substantial evidence for a role of ACE2 in regulating spermatogenesis.

Another mechanism is based on the interplay between SARS-CoV-2 and ACE2, along with changes in the intratesticular environment. Because SARS-COV-2 uses the ACE2 receptors to enter testicular cells, there are four possibilities

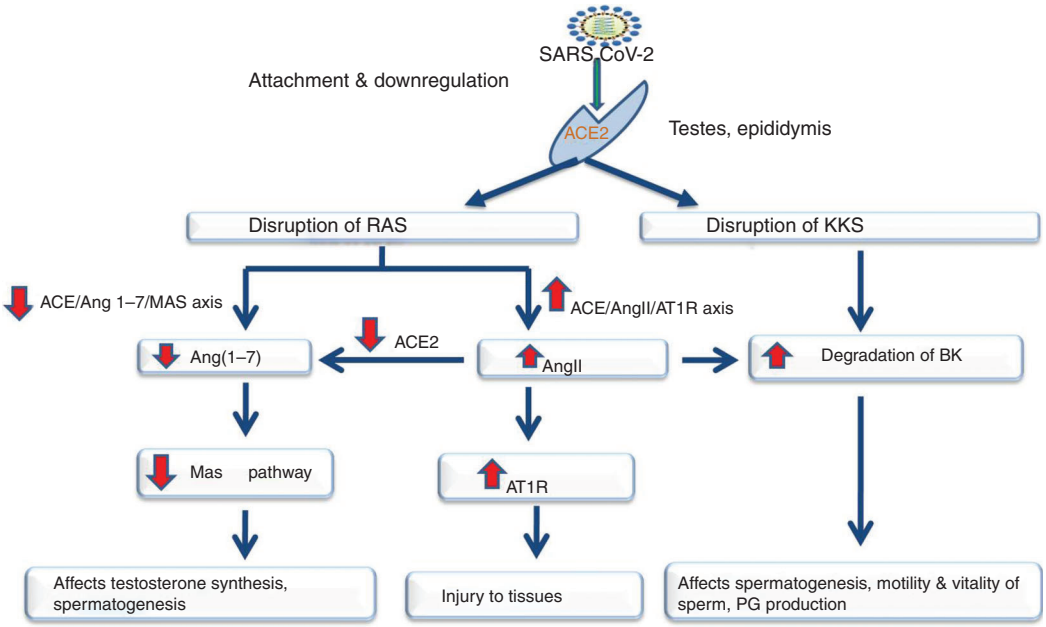

Fig. 1. Proposed mechanism by which severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) interferes with normal spermatogenesis. See text for details. ACE2, angiotensin-converting enzyme 2; RAS, renin–angiotensin system; KKS, kinin–kallikrein system; Ang, angiotensin; AT_1R: angiotensin AT_1 receptor; BK, bradykinin; PG, prostaglandin.

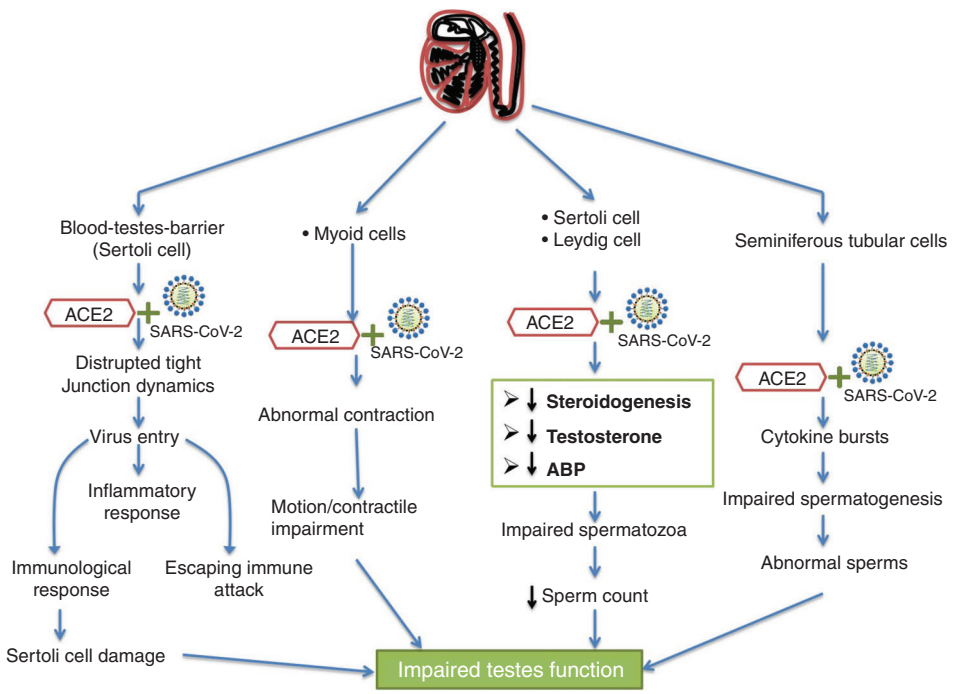

Fig. 2. Proposed mechanistic pathways by which severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) targets different compartments of the testes and impairs testicular function. In this pathway, SARS-CoV-2 is hypothesised to target cellular ACE2, the blood–testes barrier, myoid cells, Sertoli cells, Leydig cells and seminiferous tubular cells. There is a possibility that the virus may affect these targets, leading to immunological and inflammatory reactions that will result in impaired testicular functions via multiple pathways.

for the testicular disruption of spermatogenesis (Fig. 2). First, the virus may attack the Sertoli and Leydig cells, abruptly stopping spermatogenic events and causing a marked reduction in the sperm count. Second, SARS-COV-2 can target the seminiferous epithelium containing spermatogonial cells, resulting in immune and inflammatory reactions that result in cytokine-mediated bursts inside the tubules and abrupt damage to the testicular epithelium. Third, the entry of SARS-CoV-2 into Sertoli cells may lead to a disturbance in the blood–testis barrier (BTB), immunological and inflammatory responses and indirect impairment of the functional dynamics of developing spermatozoa. The BTB may not constitute a perfect barrier to viruses, as evidenced by active viral replication found in human semen during infections with the Zika (Mead *et al.* 2018), Ebola (Fischer and Wohl 2016) and Marburg (Brainard *et al.* 2016) viruses. Finally, the virus may attack the myoid cells, thereby affecting the contractile properties of tubular cells.

The third possible mechanism involves the interaction of two major cells of the testes, namely Leydig and Sertoli cells, with SARS-CoV-2. The interaction of the virus with Leydig cells may hinder the synthesis of testosterone, which is required for signalling and the overall functioning of the testes and sperm cells. Impaired FSH and LH signalling may result in aberrant functions of the testes and lead to infertility.

The proposed mechanisms above are corroborated by a recent study that reported the effect of SARS-CoV-2 infection on gonad function (Ma *et al.* 2020). That study provided the first direct evidence suggesting that SARS-CoV-2 may target the testis as the major organ of surveillance, replication, and pathogenesis (i.e. the testis is highly prone to infection, probably due to high cellular replication and turn over rate and so as under surveillance by major pathogens).

Implications of COVID-19 infection for testicular function

Patients with classical SARS had massive germ cell destruction, few or no spermatozoa in the seminiferous tubules and fibrotic basement membrane in the testes indicating testicular damage and hyperplasia (Xu *et al.* 2006). Furthermore, the Gene Set Enrichment Analysis (GSEA) method used in a recent study (Wang and Xu 2020) revealed that gene ontologies (GOs) related to the generation of male gametes, mitochondria and reproduction are downregulated, whereas cell–cell junction, immunity and immunity-related GOs are upregulated in Leydig and Sertoli cells after SARS-CoV-2 infection. Recently, Çayan *et al.* (2020) reported that serum total testosterone concentrations decreased significantly in COVID-19 patients from baseline levels. These observations were based on a cohort study conducted among COVID-19 patients categorised into three groups: Group I, asymptomatic patients; Group II, symptomatic patients admitted to an internal medicine unit; and Group III, symptomatic patients admitted to an intensive care unit (Çayan *et al.* 2020). These authors found that serum total testosterone concentrations in males are an important risk factor that significantly affect the severity of and likelihood of mortality due to COVID-19. Çayan *et al.* (2020) hypothesised that because testosterone is related to the immune system of respiratory organs, hypogonadism may contribute to the higher risk of respiratory infection. These findings suggest that COVID-19 infection dysregulates ACE2-mediated signalling in sperm cells and the testes, predisposing these subjects to the development of infertility and the production of incompetent germ cells (Wang and Xu 2020).

COVID-19 effects in the female reproductive system

Ovarian ACE2 receptors and RAS

ACE2 expression has been reported in primordial, primary, secondary and antral follicles, as well as the stroma and corpora lutea of human ovaries. *ACE2* mRNA transcripts were detected in the ovaries of reproductive-age and postmenopausal women (Reis *et al.* 2011). Thus far, there is no evidence of ACE2 expression in human oocytes. However, it has been reported that ACE2 is present in the stroma and granulosa cells and oocytes of immature rat ovaries, with ACE2 expression enhanced in antral and preovulatory follicles subjected to equine chorionic gonadotrophin treatment (Pereira *et al.* 2009). In rats, ACE2 expression was upregulated during follicle development and further increased after gonadotrophin stimulation (Pereira *et al.* 2009) and human chorionic gonadotrophin (hCG) administration (Honorato-Sampaio *et al.* 2012). As Jing *et al.* 2020 recently reviewed, *ACE2* mRNA is widely expressed in the human ovary, uterus, vagina and placenta.

Members of the local RAS in the ovary, including ACE2, are known to be involved in ovarian physiology and the regulation of follicle development, steroidogenesis, ovulation and atresia. AngII induces steroid secretion, facilitates follicle development and oocyte maturation, contributes to follicle atresia and influences ovulation (Domińska 2020). Ang-(1–7) promotes the production of oestradiol and progesterone and enhances ovulation, the resumption of meiosis (Jing *et al.* 2020) and the maturation of oocytes in women (Cavallo *et al.* 2017). The balance between AngII and Ang-(1–7) could regulate the regeneration of the endometrium (Vaz-Silva *et al.* 2009) and the activity of the myometrium (Vaz-Silva *et al.* 2012).

Binding of the SARS-CoV spike protein causes downregulation of ACE2 expression in the ovary, which remains low with persistent viral infection (Kuba *et al.* 2005; Dijkman *et al.* 2012). It can be hypothesised that, in the ovary, a decrease in ACE2 expression following SARS-CoV-2 infection may disturb the local RAS in the ovary. Disturbances in the local RAS in the ovary are associated with reproductive disorders, such as polycystic ovary syndrome (PCOS), ovarian hyperstimulation syndrome (OHS), ovarian tumours and ectopic pregnancy (Yoshimura 1997). It is unknown whether disturbances to the RAS caused by SARS-CoV-2 infection have any adverse effects on oocyte maturation and ovarian reserve.

Effects of COVID-19 in pregnant women

SARS-CoV-2 infection poses a significant risk to pregnant women and fetuses, causing premature birth (20.8%), fetal distress (26.7%), premature rupture of fetal membranes (13.0%) and Caesarean section (92.6%; Chen *et al.* 2020*b*; Ferrazzi *et al.* 2020; Li *et al.* 2020*b*; Liu *et al.* 2020; Zeng *et al.* 2020; Zhu *et al.* 2020). Moreover, approximately 2% of fetal deaths and 0.4% of neonatal deaths have been attributed to COVID-19 in South

Korea (Yee *et al.* 2020). However, Islam *et al.* (2020) recently reported that, in general, the neonatal mortality due to COVID-19 ranges between 0.5% and 2.5% worldwide. It has also been reported that ACE2 is widely expressed in the human placenta (Valdés *et al.* 2006), mainly in the syncytiotrophoblast, cytotrophoblast, endothelium and vascular smooth muscle of primary and secondary villi. During early gestation, ACE2 is expressed in the primary and secondary decidual zone, as well as in luminal and glandular epithelial cells, which further increased with advancing gestational age (Pringle *et al.* 2011). Another study suggested that pregnant mice were susceptible to SARS- CoV infection-induced renal injury because ACE2 levels in the renal tubules of pregnant mice increase by 117% compared with non-pregnant mice (Brosnihan *et al.* 2003). Thus, in terms of fertility, SARS-CoV-2-infected pregnant females are equally at risk as males.

Pregnant women are more likely to get the disease with a more severe infection than non-pregnant women. During pregnancy, fetal development increases the abdominal volume and pushes the diaphragm upwards, leading to decreased compliance of the chest wall, which eventually leads to a 20–30% reduction in functional residual capacity. To compensate for this, there is an increased tidal volume and hyperventilation. In addition, changes to the nasal mucosa mediated by progesterone during pregnancy may lead to the adhesion of the virus in the upper respiratory tract, making it difficult to clear (Toppozada *et al.* 1982; Bende and Gredmark 1999). Further, viral infection may lead to increased pulmonary vascular resistance, which may lead to pulmonary hypertension and heart failure (Nelson *et al.* 1983). According to the current statistics, a significant proportion of COVID-19 deaths are due to dyspnoea (Shi *et al.* 2020). The incidence of physical dyspnoea in the third trimester of pregnancy is 50–70% (LoMauro and Aliverti 2015). During pregnancy, the immune system also changes to T helper (TH) 2 rather than TH1 dominated, thus making the mother more susceptible to respiratory virus infection (Muallem *et al.* 2017) and autoantigen attack (Danza *et al.* 2016). Thus, it can be predicted that the SARS-CoV-2 infection will undoubtedly worsen the degree of breathing difficulties in pregnant women. Further, the expression of *ACE2* mRNA in the kidney, placenta and uterus is increased significantly during pregnancy as an adaptive feature in women to regulate blood pressure, but this can also increase the risk of contracting COVID-19 (Zhao *et al.* 2020). In this context, upregulation of ACE2 during gestation and its effect on pregnancy needs to be further investigated.

Trans-placental and -mammary transmission of SARS-CoV-2

There is an obvious concern about the effects of COVID-19 on fetal development during pregnancy as well as the manifestation of clinical symptoms in newborns after delivery. Initial studies reported negative results for the presence of the virus in babies born to pregnant women diagnosed with severe and non-severe COVID-19 infections (Chen *et al.* 2020*a*, 2020*b*). Some studies detected elevated levels of SARS-CoV-2-specific IgG and IgM, but not the viral particles, in samples collected from infants, suggesting the possibility of transplacental transfer of IgG and the presence of IgM due to possible *in utero* infection (Dong *et al.* 2020; Zeng *et al.* 2020). Similarly, Shek *et al.* (2003) reported that perinatal transmission of SARS-CoV was not detected in any of the five live-born infants who were born to pregnant women with SARS during the community outbreak in Hong Kong. However, the putative surface receptor ACE2 has been reported to be expressed in the human placenta and its expression is higher than in the lungs, suggesting the possibility of SARS-CoV-2 transmission through the transplacental route (Li *et al.* 2020*c*). However, it is unknown whether and how SARS-CoV-2 can be transmitted from the mother to the fetus. A recent comprehensive case report confirmed the vertical transmission of infection in a pregnant woman infected with COVID-19 in the third trimester of pregnancy (Vivanti *et al.* 2020). The viral load was found to be higher side in placental tissue (11.15 log10) than in amniotic fluid (2.09 log10) and maternal blood (4.87 log10) and was associated with inflammatory lesions found on the placenta. Further, the consequent viraemia and neurological manifestations in the neonate accompanied by virus-positive clinical samples confirmed the transplacental transmission of SARS-CoV-2 during the last week of pregnancy (Vivanti *et al.* 2020). Further studies are required to provide solid evidence for the vertical transmission of SARS-CoV-2 infection.

It remains unclear whether SARS-CoV-2 can be shed into breast milk and transmitted to a child through breastfeeding. Based on the available data, SARS-CoV-2 transmission may not occur through breast milk (Chen *et al.* 2020*b*; Groß *et al.* 2020; Wu *et al.* 2020). Even if there is no virus in the milk, contact transmission during breastfeeding should be taken into account and, to minimise the risk of transmission to the neonate, proper quarantine measures need to be enforced for the mother. The United Nations International Children's Emergency Fund (UNICEF) suggests that COVID-19-positive mothers may continue breastfeeding while using precautions, such as wearing a mask and washing their hands before and after feeding (UNICEF 2020).

Sex differences in COVID-19 infections

Global data suggest that there is a sex difference in mortality from COVID-19. It has been reported that men have been more severely affected than women during the COVID-19 pandemic globally (Chamekh and Casimir 2020). According to statistics available in an open dataset, 2.4-fold more men have died during the recent pandemic than women (Jin *et al.* 2020). Possible biological reasons for this sex difference that have been proposed by various researchers are summarised in Table 1.

Sex-disaggregated data for COVID-19 infections

As per the report from Global Health 50/50 (2020), total sex-disaggregated data for COVID-19 infections are available for 75 countries (Fig. 3). The data indicate that the number of confirmed cases and fatality rates are higher for males, mostly from Asia, America and Africa. In males, the proportion of highest confirmed cases (80%) has been reported in Cambodia and the highest fatality rate (77%) has been reported in Chad. Other major countries with higher fatality in males include Bangladesh (77%), Thailand (76%), Malawi (76%), Nigeria (75%), Afghanistan (75%), Burkina Faso (75%) and Pakistan (74%).

In the US, although the number of confirmed cases is higher (52%) in females, the mortality rate is higher (54%) among males. The data from India indicated that the mortality rate is higher in males than females (64% vs 36%).

In most European countries, a greater number of females have tested positive for COVID-19 than males. The highest numbers of COVID-19-positive females were reported from Ukraine (60%), Moldova (59%), Wales (56%), Latvia (56%), Scotland (55%), Belgium (55%) and Portugal (55%), and the highest female mortality was reported in Vietnam (63%), Slovenia (54%), Canada (53%), Estonia (53%), Australia (52%), Finland (51%) and Belgium (51%). In European countries, the number of confirmed cases and mortality rates are rising in the female rather than male population, which indicates the need for the collection of more relevant data, more detailed analysis and a greater understanding of the virus.

Table 1. Plausible biological reasons for sex differences in COVID-19 infectivity

Factor	Sex differences	References
Immunity	Stronger innate immune response in females than males	Jaillon *et al.* (2019)
Genetics	X-linked genes harbour more immune-related genes	Schurz *et al.* (2019)
High-risk factors	Smoking, alcohol consumption, tobacco chewing and occupational exposures are less prevalent among women	Global Health 50/50 (2020)
Behavioural and social factors	Women show more interest than men in following hand hygiene practices and pursuing preventive care	Johnson *et al.* (2003), Bertakis *et al.* (2000)
ACE2	The testes have much higher levels of ACE2 than the ovaries	Chen *et al.* (2020*a*)
Comorbid conditions for COVID-19	The prevalence of conditions like hypertension, diabetes, chronic kidney diseases and cancer is lower among women than men	Wu and McGoogan (2020)
Hormone factors	17β-Oestradiol downregulates ACE2 cellular receptors; testosterone suppresses the innate immune system in general	Gianzo *et al.* (2018), Stelzig *et al.* (2020)

Differential expression of ACE2 in males and females

The greater susceptibility of males towards COVID-19 infection than females may be due to increased expression of ACE2 receptors in the male reproductive system (Robinson *et al.* 2020). Higher ACE2 expression in males may be attributed to the virus membrane fusion protein TMPRSS2 being an androgen-responsive gene (Kron *et al.* 2017; Asselta *et al.* 2020), in line with previous studies on H1N1 and H7N9 influenza (Cheng *et al.* 2015). Moreover, only gonadotrophin-dependent expression of ACE2 has been reported in the ovaries (Robinson *et al.* 2020). Furthermore, some reports suggest that the presence of ACE2 receptors in ovarian and uterine tissues did not facilitate SARS-CoV infection (Ding *et al.* 2004). There are also reports proving the cardiovascular protective effect of circulating ACE2 and explain the relative protection of females against heart diseases compared with males. Together, this evidence seems to indicate that the putative sex predisposition to COVID-19 infection, with men being more susceptible, may be reflective of a peculiar ACE plasma profile (Ciaglia *et al.* 2020).

In a study in rats, Dalpiaz *et al.* (2015) reported that ACE and ACE2 activity was greater in male than female rats. Further

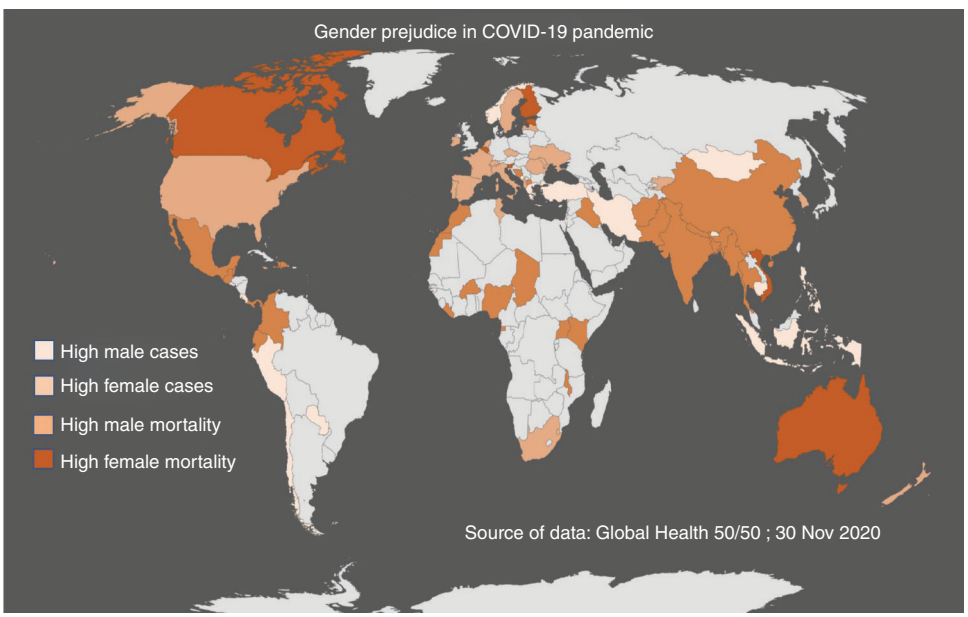

Fig. 3. Sex-disaggregated data of COVID-19 infections across the globe. Prepared using data available from Global Health 50/50 (2020). Updated on 30 November 2020.

gonadectomy in both sexes promoted opposite responses in haemodynamic and cardio vascular parameters which are under the influence of ACE2. Thus, ACE2 activity is said to be sex steroid dependent. There is ample evidence that the expression of AngII and the angiotensin AT_1 receptor (classical RAS components) are higher in males, whereas expression of the AT_2 receptor and Ang(1–7), non-classical RAS components, is higher in females (Sullivan *et al.* 2015). In another study in rats, ACE2 expression was markedly reduced with aging in both sexes; although there was no sex-related difference in ACE2 in young adult and middle-aged groups, ACE2 content was significantly higher in old female than male rats (Sullivan *et al.* 2010).

Hypothetical role of 17β-oestradiol in curbing COVID-19 infection

The primary female sex hormone oestrogen, in both natural and synthetic forms, has an immune-suppressive effect at high concentrations and immunostimulatory activity at low concentrations (Klein and Flanagan 2016). 17β-Oestradiol (E2) is a potent anti-inflammatory hormone and the treatment of female mice with E2 reduces morbidity due to Influenza A virus infection compared with experimentally infected placebo-treated ovariectomised female C57BL/6 mice (Robinson *et al.* 2014). Oestrogen replacement treatment for 3 weeks in ovariectomised transgenic hypertensive (mRen2) rats resulted in reductions in plasma and tissue ACE activity (aorta and kidney), as well as circulating AngII concentrations, whereas circulating Ang-(1–7) concentrations were increased (Brosnihan *et al.* 1999). Oestrogen administration increases the expression a disintegrin and metalloproteinase (ADAM) 17 and ADAM10, two putative ectodomain shedders in atherosclerosis (Zhou *et al.* 2020). This suggests a protective role for oestrogen against cardiovascular events in females, a mechanism potentially accounting for the observed COVID-19 infection sex disparity. Conversely, the decrease in oestrogen in postmenopausal women affects TMPRSS2 expression, the gene being responsive to oestrogens (Baena *et al.* 2013).

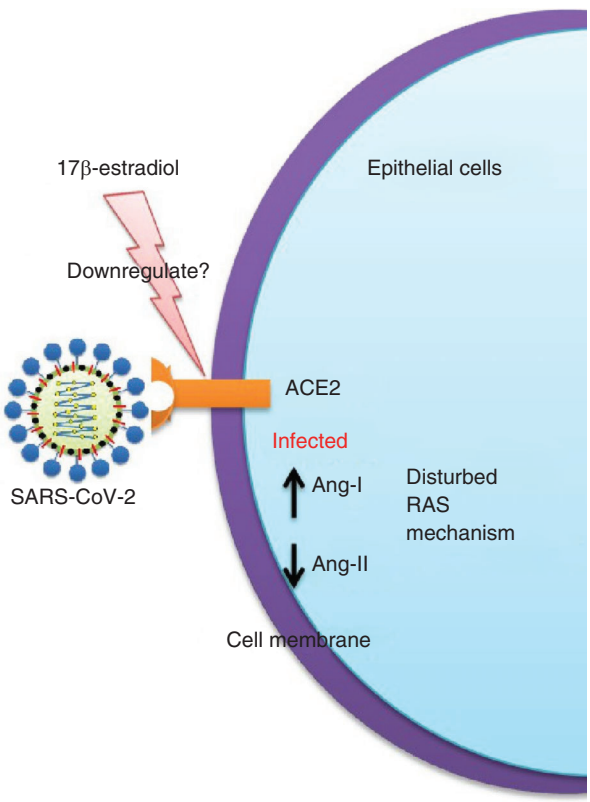

Fig. 4. Effect of SARS-CoV-2 infection on epithelial cells. A decrease in angiotensin-converting enzyme (ACE) 2 expression following SARS-CoV-2 infection may disturb cellular renin–angiotensin system (RAS) mechanisms. 17β-Oestradiol downregulates membrane ACE2 receptors, thereby hindering SARS-CoV-2 infectivity.

The presence of E2 does not affect virus replication but rather varies the production of chemokines, the pulmonary recruitment of neutrophils and the cytokine responses of virus-specific CD8 T cells to protect females against severe influenza (Robinson *et al.* 2011). An *in vivo* study using a mouse model of SARS-CoV infection (Channappanavar *et al.* 2017) was used to demonstrate that female mice are less susceptible to SARS-CoV infection than male mice. Moreover, Channappanavar *et al.* (2017) found that the removal of the testes did not affect disease outcomes in male mice, whereas ovariectomy or treating female mice with the oestrogen receptor antagonists ICI-182 and ICI-780 resulted increased mortality to SARS-CoV infection, signifying that oestrogen signalling protects female mice from lethal SARS-CoV infection. In light of the recent pandemic situation, the same research team has stated that this could also be the reason for the male susceptibility to COVID-19 (Iyer *et al.* 2020).

SARS-CoV-2 and SARS-CoV attack cells via ACE2, and it has been shown that circulating ACE2 levels are higher in men than in women and in patients with diabetes or cardiovascular diseases (Patel *et al.* 2013). In a study conducted recently using differentiated normal human bronchial epithelial (NHBE) cells treated with E2 *in vitro*, ACE2 expression was downregulated but the underlying molecular mechanisms have yet to be elucidated (Fig. 4; Stelzig *et al.* 2020). Conversely, administration of exogenous oestradiol increased *ACE2* mRNA expression and activity in the kidney and uterus of pregnant animals (Neves *et al.* 2008), indicating oestrogen could be a regulator of ACE2, although other hormones could also be involved. In atrial tissue slices prepared from male donors, oestrogen increased the amount of *ACE2* mRNA (Brosnihan *et al.* 2008). These studies reveal the tissue-specific regulation of ACE2 receptor genes by E2.

Conclusion

COVID-19 affects the reproductive system of both males and females. The mechanisms and pathways by which the virus affects gonad function, and the consequences, are not yet clear. The robust and widespread distribution of ACE2 in the male reproductive system predisposes males to the entry, localisation, replication and pathogenesis of the virus. SARS-CoV-2 may infect the ovary, uterus, vagina and placenta through the ubiquitous expression of ACE2, and can disturb female reproductive function, resulting in infertility, menstrual disorder and fetal

distress. COVID-19 is likely to have long-term effects on male and female reproductive systems, as well as fertility status. Integration of mass data, genomics, proteomics and metabolomics should be used to understand the deleterious effects of this virus. Therefore, future studies are warranted to elucidate the different pathways and cellular responses to virus infection in both the male and female reproductive system. Understanding the key mechanistic pathways will help design suitable strategies for the effective management and treatment of COVID-19 and its associated reproductive impairment.

Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Declaration of funding

No funding was availed for the work.

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Handling Editor: Geraldine Hartshorne